Suppl. Figure 1
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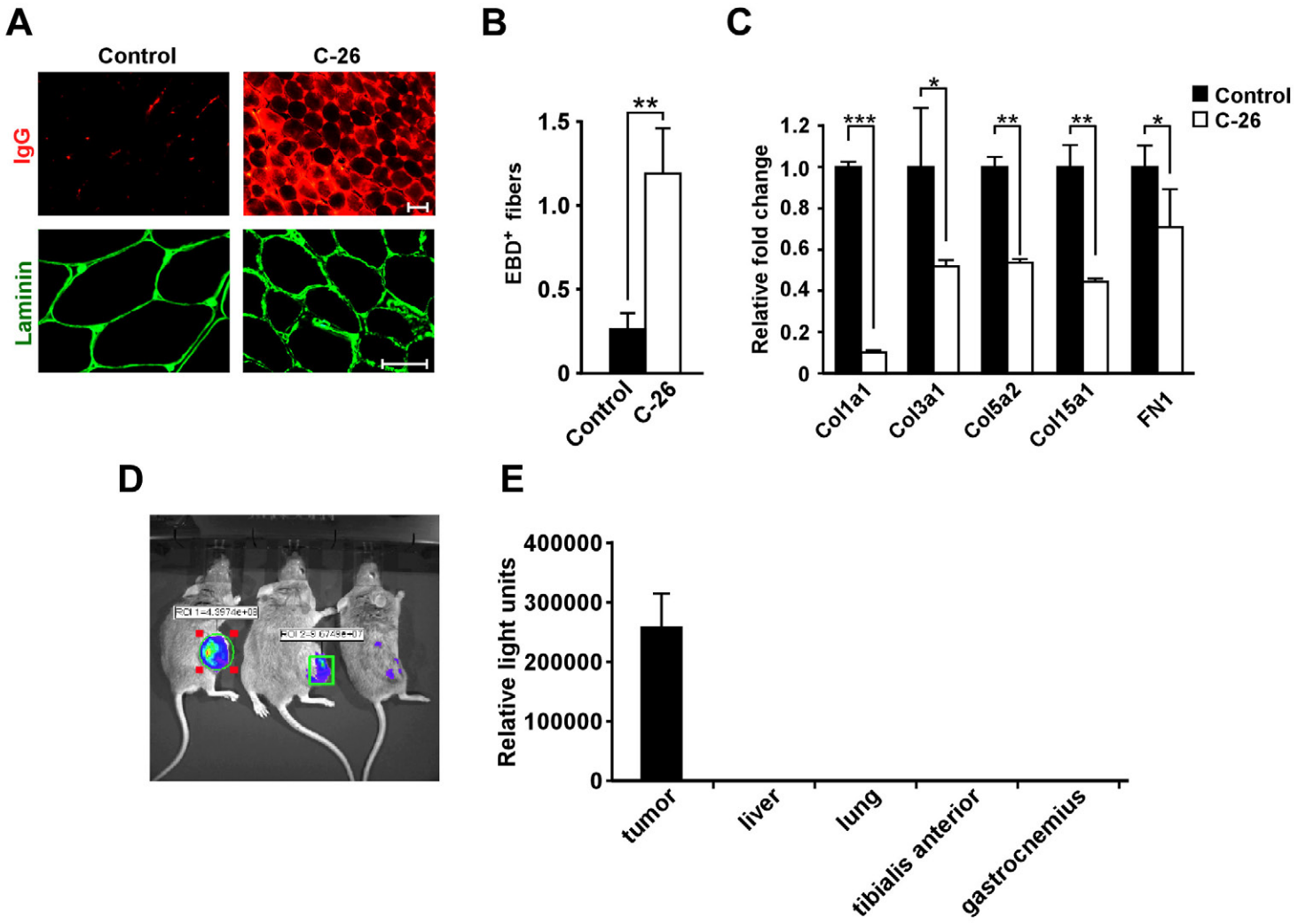

Figure S1. Alterations in the muscle sarcolemma and basal lamina result from systemic tumor factors. (**A**) Cross sections prepared from gastrocnemius muscles of control and tumor mice were stained with mouse IgG (upper panels) or α-laminin (lower panels). Scale bars, 50 μm. (**B**) Evans blue dye were injected in control and tumor mice, and cross sections were subsequently examined and quantitated for damaged myofibers. (**C**) Muscles from **A** above were analyzed by real-time RT-PCR for extracellular matrix genes including collagen 1a (Col1a1), collagen 3a (Col3a1), collagen 5a (Col5a2), collagen 15a (Col15a1), and fibronectin (FN1). (**D**) Mice were injected with C-26 tumor cells stably expressing a luciferase reporter gene. After 3 weeks of implantation, mice were injected with luciferin substrate and reporter activity was visualized by a live imaging system. (**E**) Tumor and other organs indicated were harvested from mice in **D**, and subsequently analyzed for reporter activity by luciferase assays. Asterisks indicate $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).

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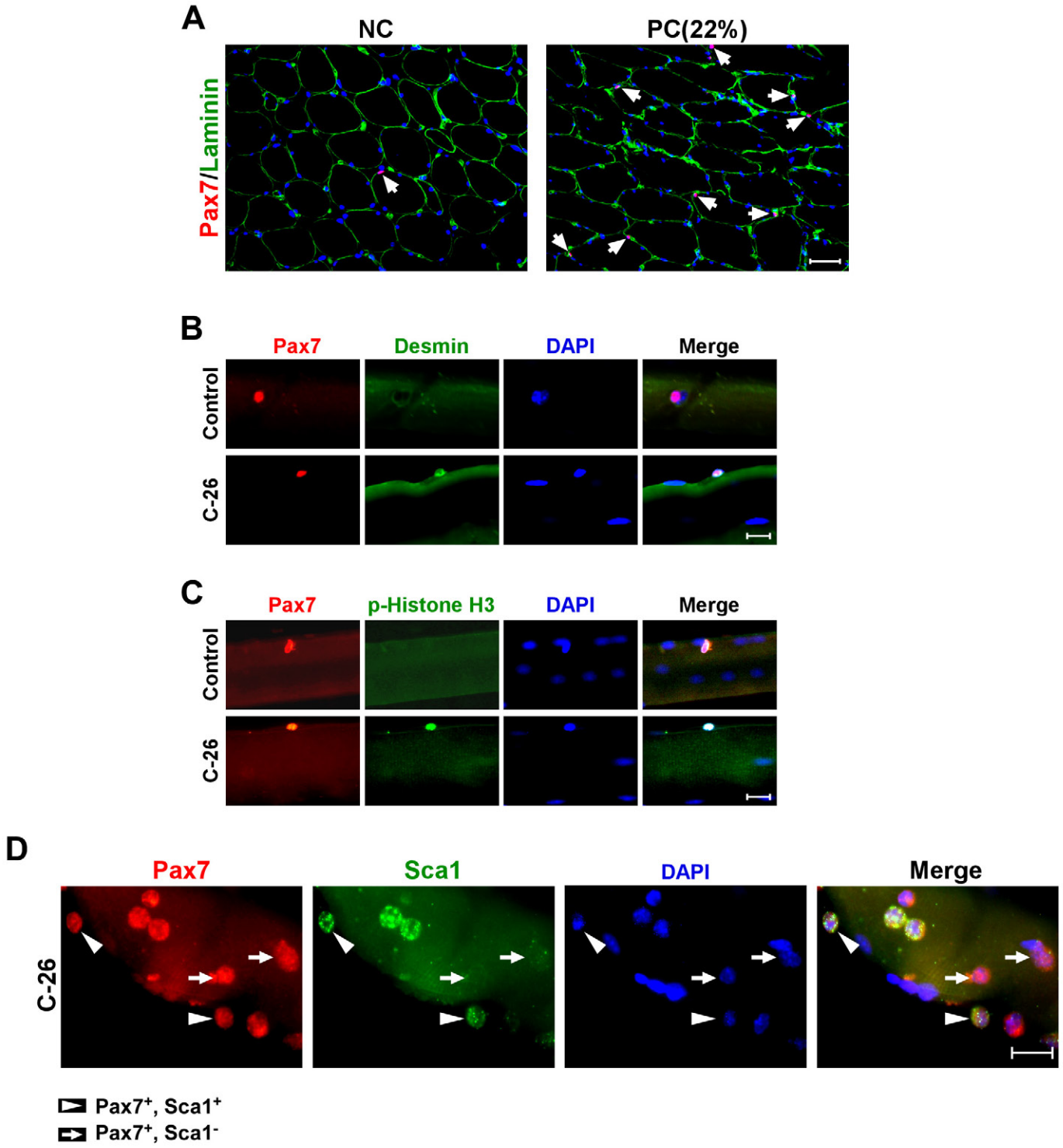

Figure S2. Cancer cachexia is associated with the increase and activation of satellite cells. (**A**) Representative staining of Pax7 (red), α-laminin (green), and DAPI (blue) of muscle sections from a non-cancer control (NC) and a pancreatic cancer (PC) patient. Arrows denote $Pax7^+$ cells. Scale bar, 50 μm. (**B**) Single fibers isolated from control and C-26 tumor mice were immunostained with Pax7 (red), desmin (green), and DAPI (blue). Scale bar, 20 μm. (**C**) Single fibers from the same mice as in **B** were immunostained with Pax7 (red), phospho-histone H3 (green), and DAPI (blue). Merged images are shown to denote the specificity of the staining. Scale bar, 20 μm. (**D**) Magnified images from Figure 2J.

Suppl. Figure 3
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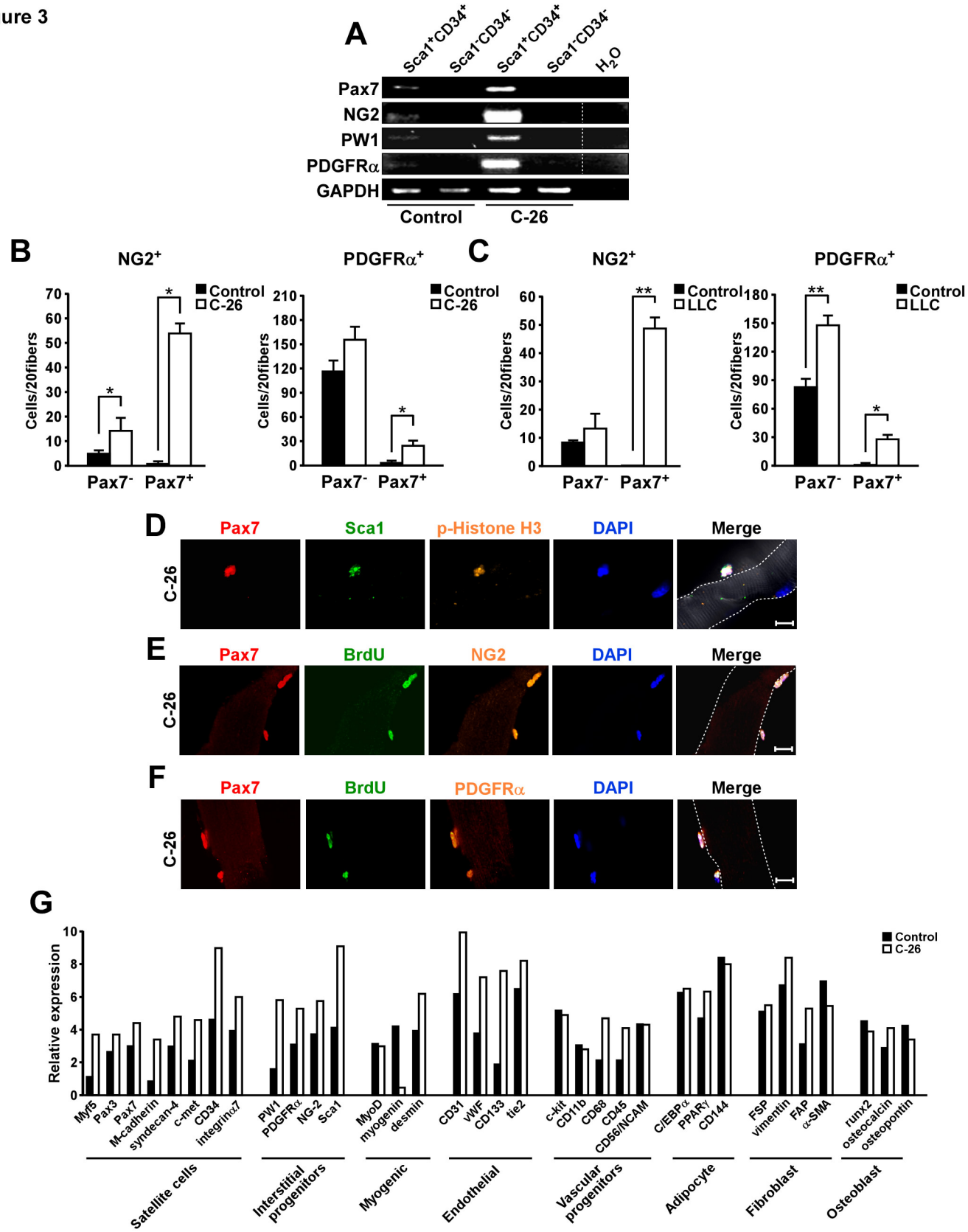

Figure S3. Cancer cachexia is associated with the increase and activation of multiple populations of muscle progenitor cells. (**A**) RNA was prepared from sorted Sca1$^+$, CD34$^+$ and Sca1$^-$, CD34$^-$ cells (as negative control) and RT-PCR was performed for Pax7 and interstitial cell markers NG2, PW1, and PDGFRα. GAPDH serves as loading control. (**B-C**) Single fibers isolated from (**B**) control and C-26 tumor mice or (**C**) control and LLC tumor mice were co-immunostained with Pax7/NG2 or Pax7/PDGFRα. Quantitation was performed for NG2$^+$Pax7$^+$ (left graph) and PDGFRα$^+$Pax7$^+$ (right graph) cells. (**D**) Single fibers from tumor mice were immunostained with Pax7 (red), Sca1 (green) and phospho-histone H3 (orange). Nuclei were counterstained with DAPI (blue). (**E**) Single fibers from tumor mice were immunostained with Pax7 (red), BrdU (green) and NG2 (orange). Nuclei were counterstained with DAPI (blue) and images were merged to indicate specificity. (**F**) Single fibers from control and tumor mice were immunostained with Pax7 (red), BrdU (green), PDGFRα (orange) and DAPI (blue). Scale bars, 25 μm. Merged images are shown to indicate specificity. (**G**) Graph represents expression levels of genes in sorted Sca1$^+$, CD34$^+$ cells from muscles of control and C-26 tumor mice using NanoString custom arrays. Relative expression was determined by normalizing with the average expression values (geometric mean) of four housekeeping genes. Classification of genes was assigned through the identification of cell markers as described in the literature. Results were repeated from two independent experiments.

Suppl. Figure 4
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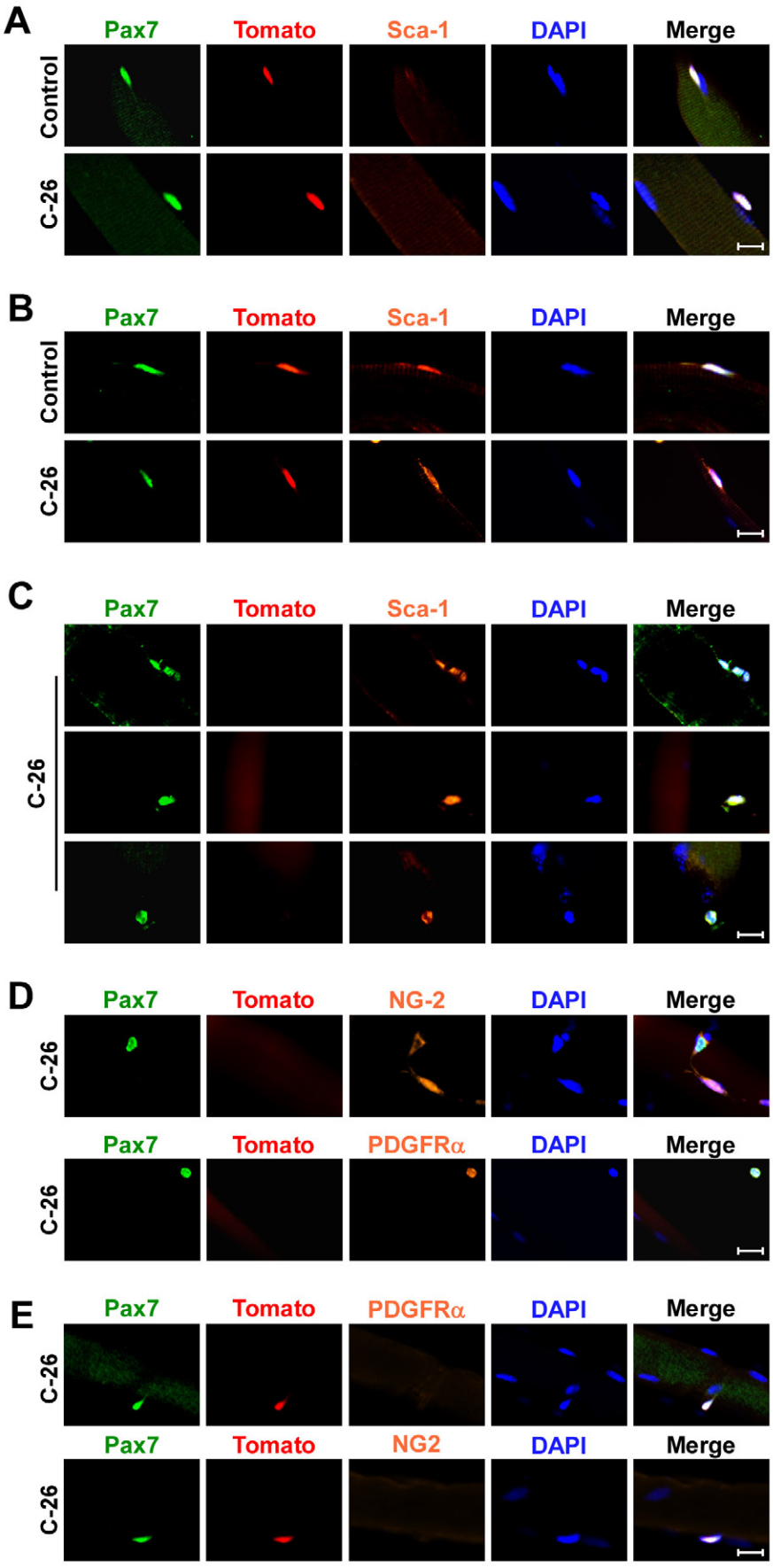

Figure S4. Increase in Pax7^{+}, Sca1^{+} cells during cancer cachexia derives mainly from non-satellite cell lineage. Myofibers were isolated from *Pax7-Cre^{ER}; Rosa26-Tomato* reporter mice that were induced with tamoxifen before tumor implantation, and subsequently immunostained for Pax7, Sca1, and DAPI. Pictures represent: (**A**) Pax7^{+}, Tomato^{+}, Sca1^{-} satellite cells in both non-tumor and tumor conditions, (**B**) Pax7^{+}, Tomato^{+}, Sca1^{+} satellite cells in both non-tumor and tumor conditions, and (**C**) Pax7^{+}, Tomato^{-}, Sca1^{+} cells detected in tumor bearing mice. (**D**) Pax7^{+}, Tomato^{-} cells on muscles from tumor bearing mice were analyzed with non-satellite cell markers NG2 or PDGFRα. (**E**) Pax7^{+}, Tomato^{+} satellite cells were analyzed for NG2 and PDGFRα expression in C-26 tumor bearing mice. Scale bars, 20 mm.

Suppl. Figure 5
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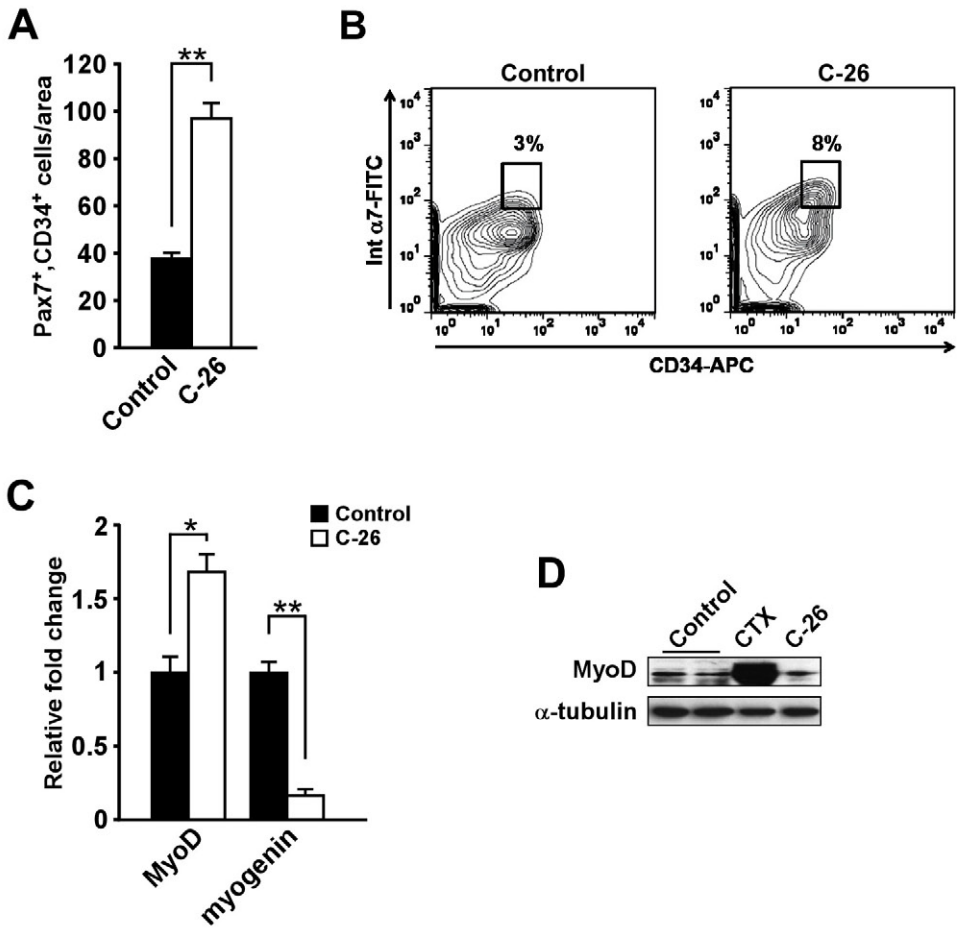

Figure S5. Cancer cachexia is associated with an aberrant commitment to the myogenic program. (**A**) Mononuclear cells were isolated from control and cachectic muscles, and following two rapid pre-plating steps, attached cells were cultured on gelatin-coated plates overnight. The following day, cells were fixed and immunostained for satellite cell markers, Pax7 and CD34. Graph shows average number of double positive cells per area. Results were repeated from three independent experiments. (**B**) Muscle mononuclear cells were prepared from control and C-26 mice and analyzed for satellite cells by FACS. Sorted cells negative for c-kit, CD11b, CD31, CD45, and Sca1 (not shown) were analyzed by FACS for expression of integrin-α7 and CD34. Area indicated in rectangles represents the % increase of integrin-$\alpha7^+$, $CD34^+$ cells in cachectic muscle. Graphs are representative of three independent experiments. (**C**) $CD34^+$, integrin-$\alpha7^+$, $Sca1^-$, $CD31^-$, $CD45^-$, $CD11b^-$, satellite cells were sorted and analyzed by real-time RT-PCR for MyoD and myogenin. (**D**) Myogenic cells were FACS purified similar to that described in **C**, and westerns were performed probing for MyoD, using tubulin as loading control. Note that although MyoD mRNA is slightly increased in tumor bearing mice, this same increase is not seen at the protein level. To verify our results, additional control was added in **D**, where FACS sorting from hindlimb muscles was repeated with mice undergoing acute muscle injury using cardiotoxin (CTX). α-tubulin was used as loading control. Asterisks indicate $p < 0.05$ (*) and $p < 0.01$ (**).

Suppl. Figure 6
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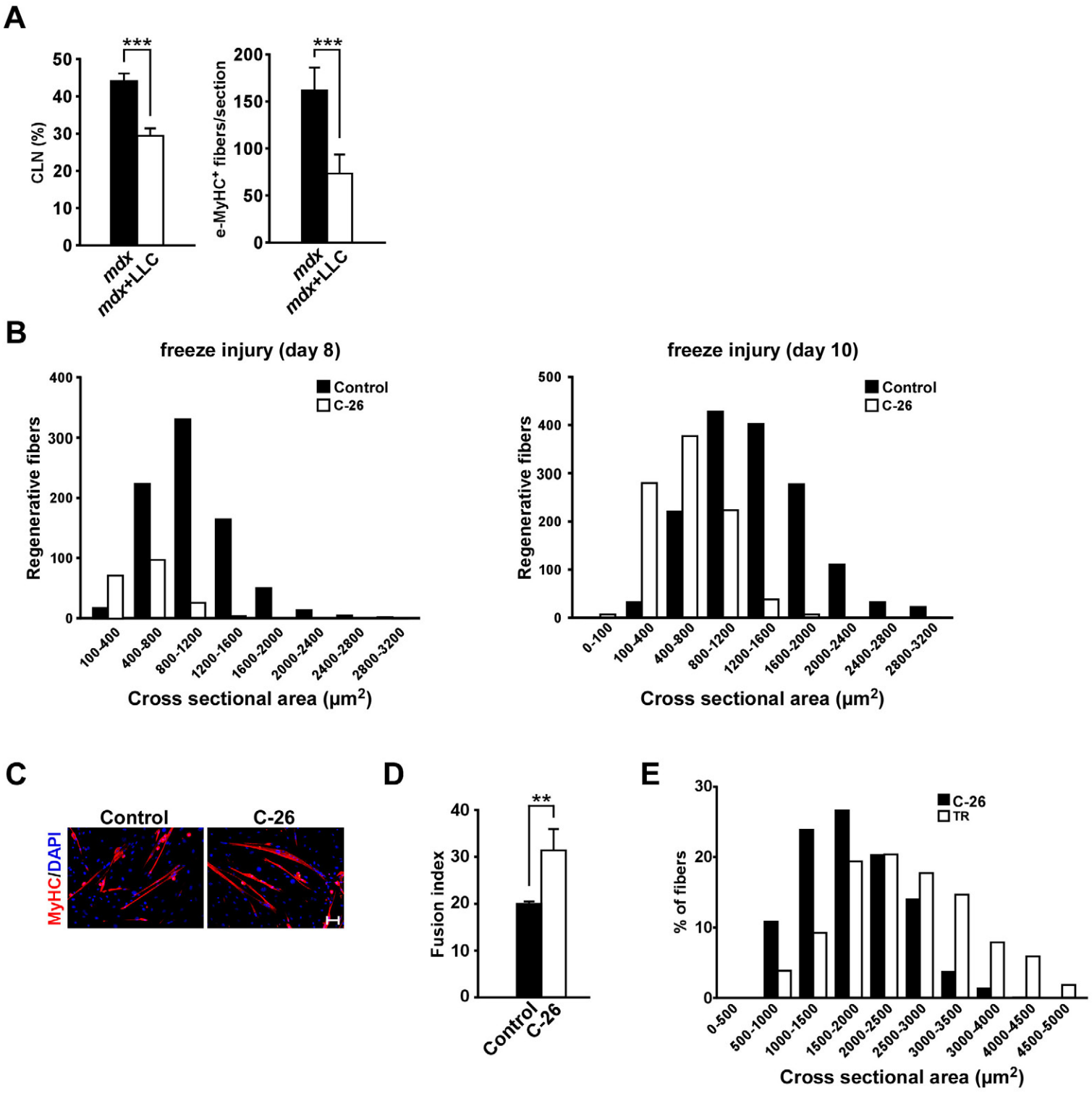

Figure S6. Muscle regeneration is impaired in cancer cachexia. (**A**) Gastrocnemius muscles from control *mdx* or LLC tumor bearing *mdx* mice were frozen, sectioned and analyzed. Left: randomly selected areas from H&E stains were quantitated for the percentage of CLN; Right: randomly chosen cross sections stained with e-MyHC and Col IV were quantitated for e-MyHC$^+$ fibers. (**B**) Cross sectional area measurements made from TA muscles that were isolated 8 (left graph) and 10 (right graph) days after freeze injury in control and C-26 tumor mice. (**C**) Mononuclear cells from hindlimb muscles of control and tumor mice were cultured directly in differentiation medium immediately following their isolation. Cells were then fixed and immunostained for MyHC and nuclei counterstained for DAPI. Scale bar, 25 μm. (**D**) Fusion index measurement from the result in **C**. Results were repeated from five independent experiments. (**E**) Cross sectional area measurements made from GAST muscles from C-26 tumor or tumor removed mice (TR). Asterisks denote $p < 0.01$ (**), $p < 0.001$ (***).

Suppl. Figure 7
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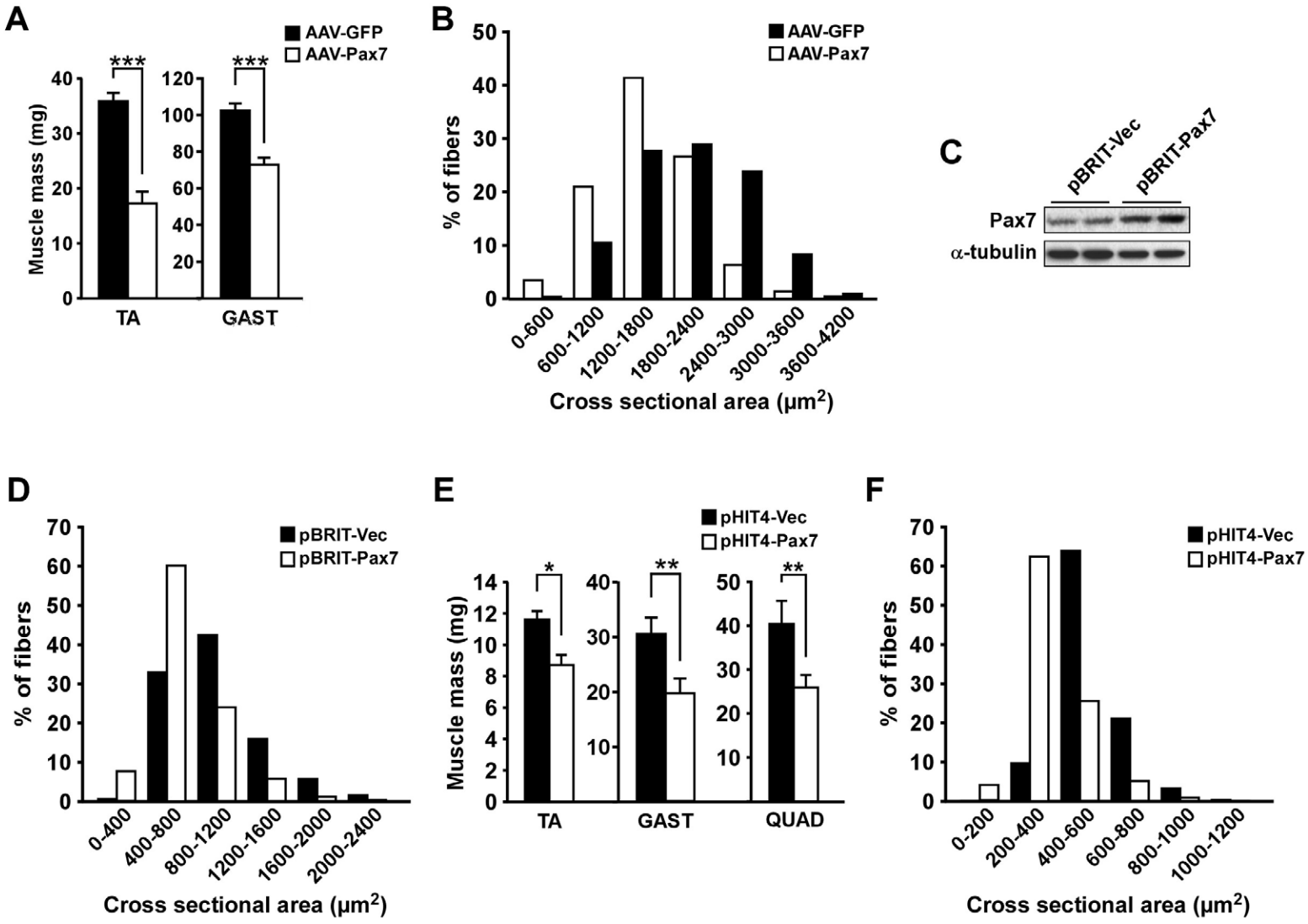

Figure S7. Pax7 regulates muscle wasting in cancer cachexia. (**A**) TA and GAST muscle mass measured from C-26 tumor bearing mice injected with AAV expressing GFP control (n=5) or AAV expressing Pax7 (n=6). (**B**) Cross sectional area measurements of GAST muscles from C-26 tumor bearing mice injected with AAV control or Pax7-expressing AAV. (**C**) Hind limbs from neonatal mice were injected with pBRIT-Vec or pBRIT-Pax7 retroviruses and at P15 primary myoblasts were prepared and probed for Pax7 by western analysis. α-tubulin was used as a loading control. (**D**) Cross sectional area measurement of GAST muscles from mice in **C**. (**E**) TA, GAST and QUAD muscle masses were recorded in P15 mice following the injection of retroviruses, pHIT4-Vec (n=5) and pHIT4-Pax7 (n=5). (**F**) Cross sectional area measurements of GAST muscles from mice described in **E**. Asterisks denote $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).

Suppl. Figure 8
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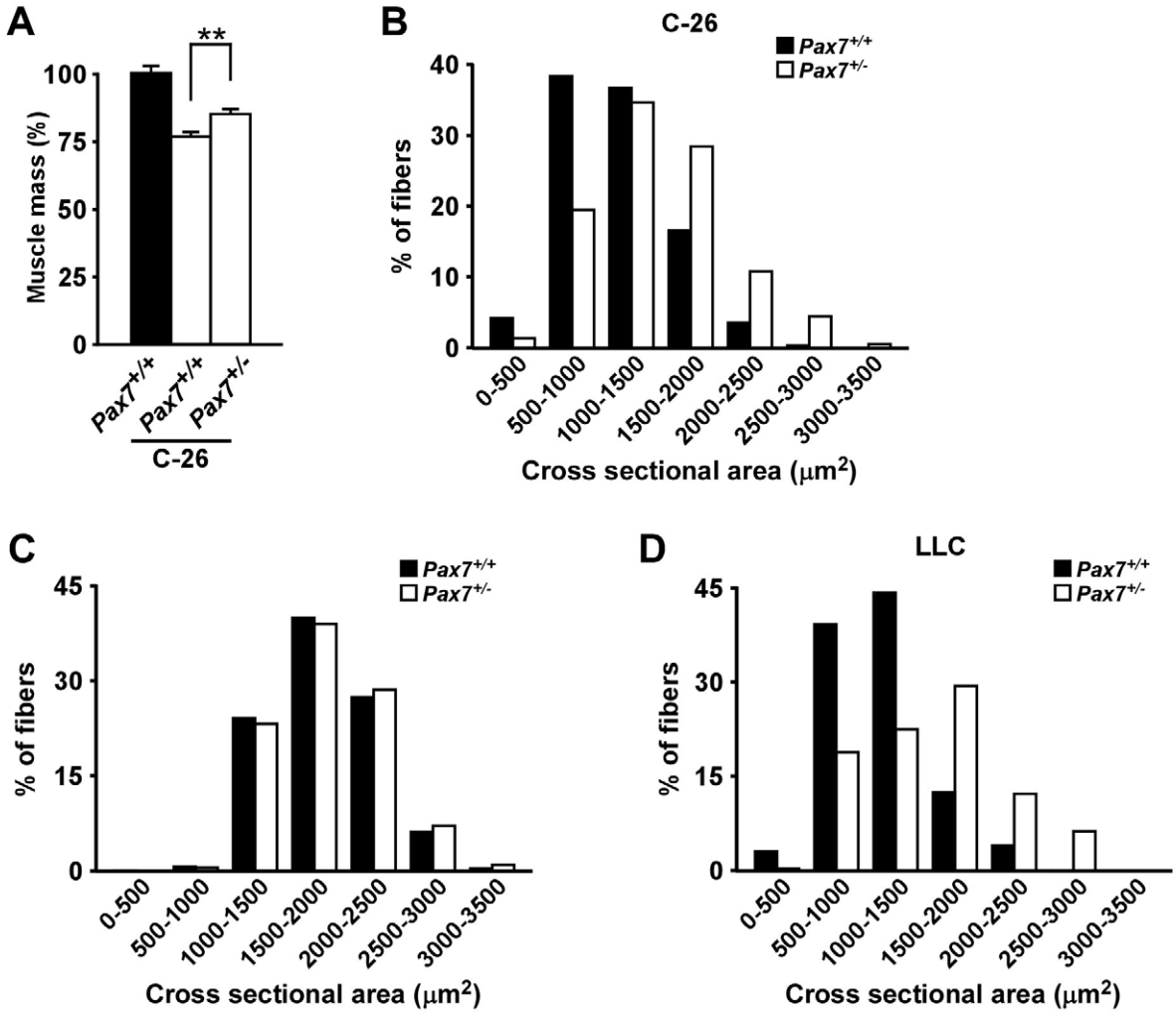

Figure S8. *Pax7*$^{+/-}$ mice are protected against cancer-induced muscle wasting. (**A**) GAST muscle mass from C-26 tumor bearing *Pax7*$^{+/+}$ (n=10) and *Pax7*$^{+/-}$ (n=10) mice was measured and normalized to that from age and genotype matched non-tumor mice, which were set at 100%. Results were repeated from four independent experiments. (**B-D**) Cross sectional area measurements made from GAST muscles from *Pax7*$^{+/+}$ and *Pax7*$^{+/-}$ mice either bearing C-26 tumors (**B**), no tumors (**C**), or LLC tumors (**D**). Asterisks denote $p < 0.01$ (**).

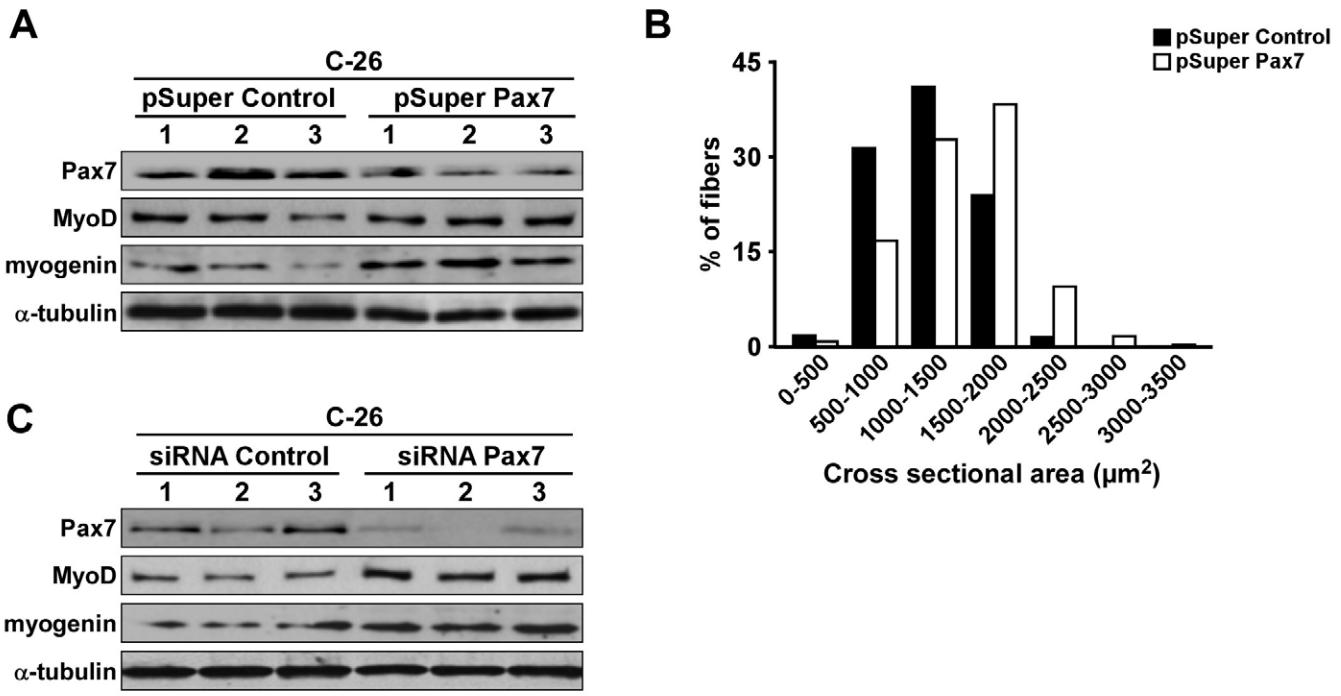

Figure S9. Pax7 promotes muscle wasting in cancer cachexia by regulating the MyoD/myogenin regulatory axis. (**A**) C-26 tumor mice were intramuscularly injected with pSuper retroviruses expressing either control (n=8) or Pax7 shRNA (n=10), and levels of Pax7 were subsequently verified by western blot, along with levels of downstream factors, MyoD and myogenin. Blots were stripped and reprobed for α-tubulin as a loading control. (**B**) Cross sectional area measurements made from muscles from C-26 tumor bearing mice in **A**. (**C**) Western blots probing for Pax7, MyoD, and myogenin in muscles from C-26 tumor mice treated with systemic delivery of control (n=5) or siRNA targeting Pax7 (n=5). Results are representative of two independent experiments.

Suppl. Figure 10
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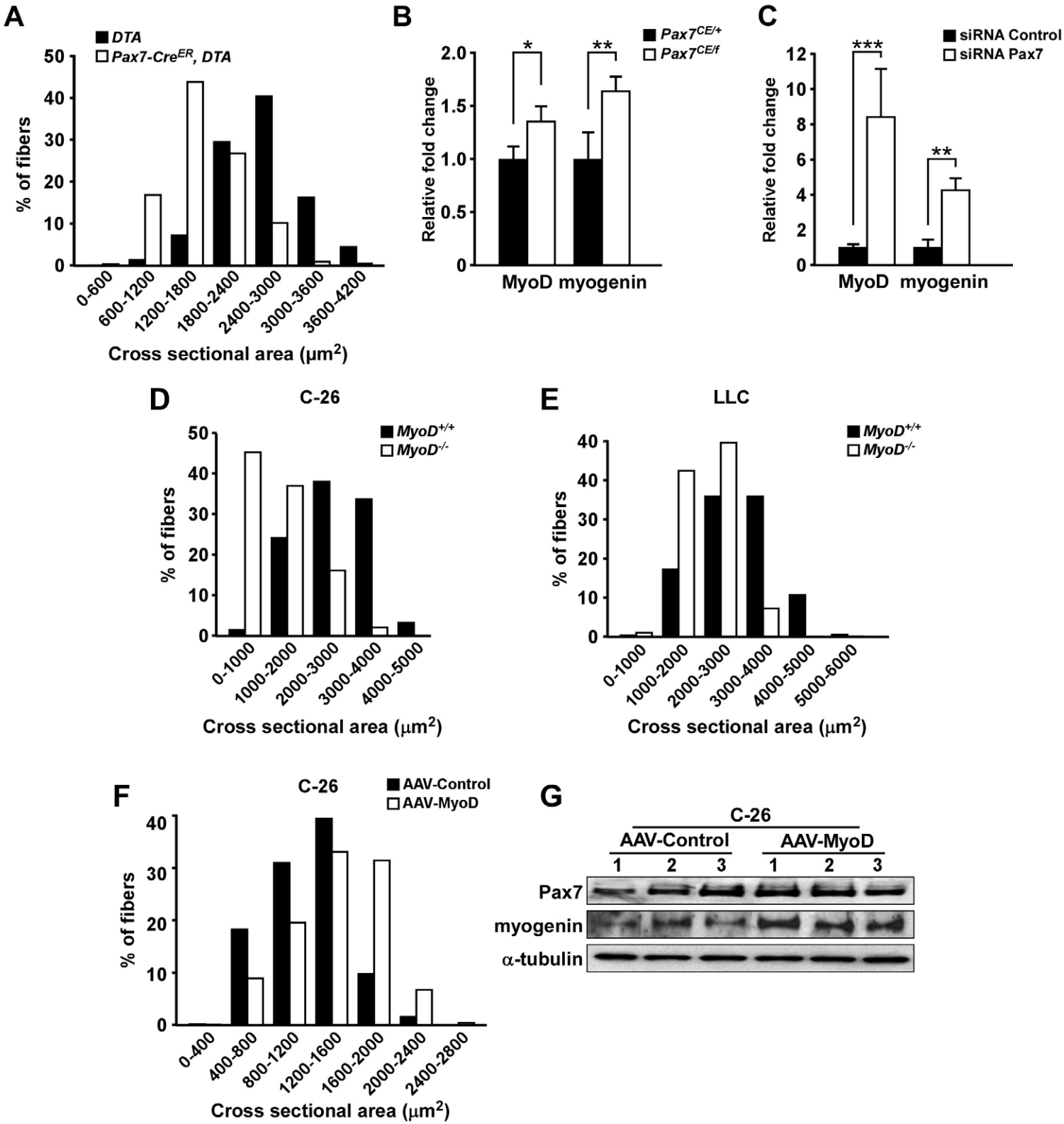

Figure S10. shRNA and siRNA knockdown of Pax7 rescues muscle mass in cancer cachexia. (**A**) Cross sectional area measurements made from muscles from LLC tumor bearing where $Pax7^+$ cells were conditionally depleted with DTA. (**B**) GAST muscles from *Pax7*$^{+/f}$ and *Pax7*$^{f/CE}$ tumor mice were analyzed by real time RT-PCR for MyoD and myogenin. (**C**) Similar RT-PCR analysis for MyoD and myogenin was performed with GAST muscles from tumor mice treated with control or siRNA against Pax7. (**D**) Cross sectional area measurements made from GAST muscles of C-26 tumor bearing *MyoD*$^{+/+}$ and *MyoD*$^{-/-}$ mice. (**E**) Cross sectional area were measured from GAST muscles from LLC tumor bearing *MyoD*$^{+/+}$ and *MyoD*$^{-/-}$ mice. (**F**) Cross sectional area measurements of muscles from tumor mice expressing AAV-control or AAV-MyoD. (**G**) Muscles from tumor mice intramuscularly injected with AAV-control or AAV-MyoD were analyzed by western blots probing for Pax7, myogenin, and α-tubulin. Asterisks denote $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).

Suppl. Figure 11
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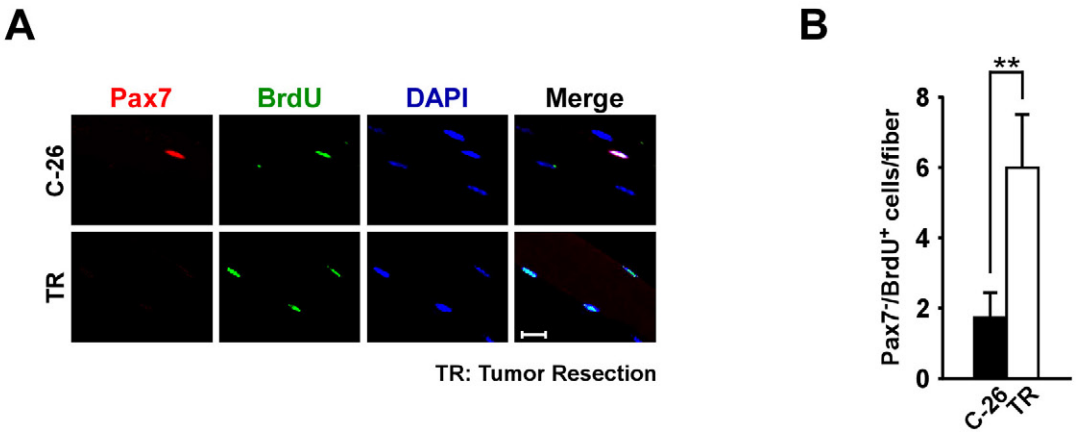

Figure S11. Tumor resection rescues myogenic terminal differentiation. (**A**) Single fibers from C-26 tumor bearing mice and tumor resected mice were stained for Pax7 (red), BrdU (green) and DAPI (blue). Merged pictures were shown to denote specificity. Scale bar: 20μm. (**B**) Graph represents quantitation of $Pax7^-$, $BrdU^+$ myonuclei per fiber as described in **A**.

Suppl. Figure 12
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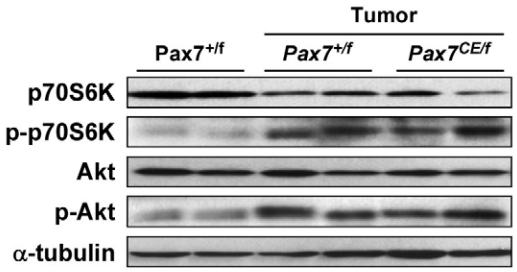

Figure S12. Pax7-mediated muscle wasting in cancer is independent from the regulation of atrogenes and Akt/mTOR activities. Muscle cell extracts were prepared from *Pax7*$^{+/f}$ and *Pax7*$^{f/CE}$ tumor mice and westerns were performed probing for total and activated forms of Akt and p70S6K. α-tubulin was used as a loading control. Westerns shown in duplicate are representative of results observed with a minimum of n=4 muscle samples.

Suppl. Figure 13
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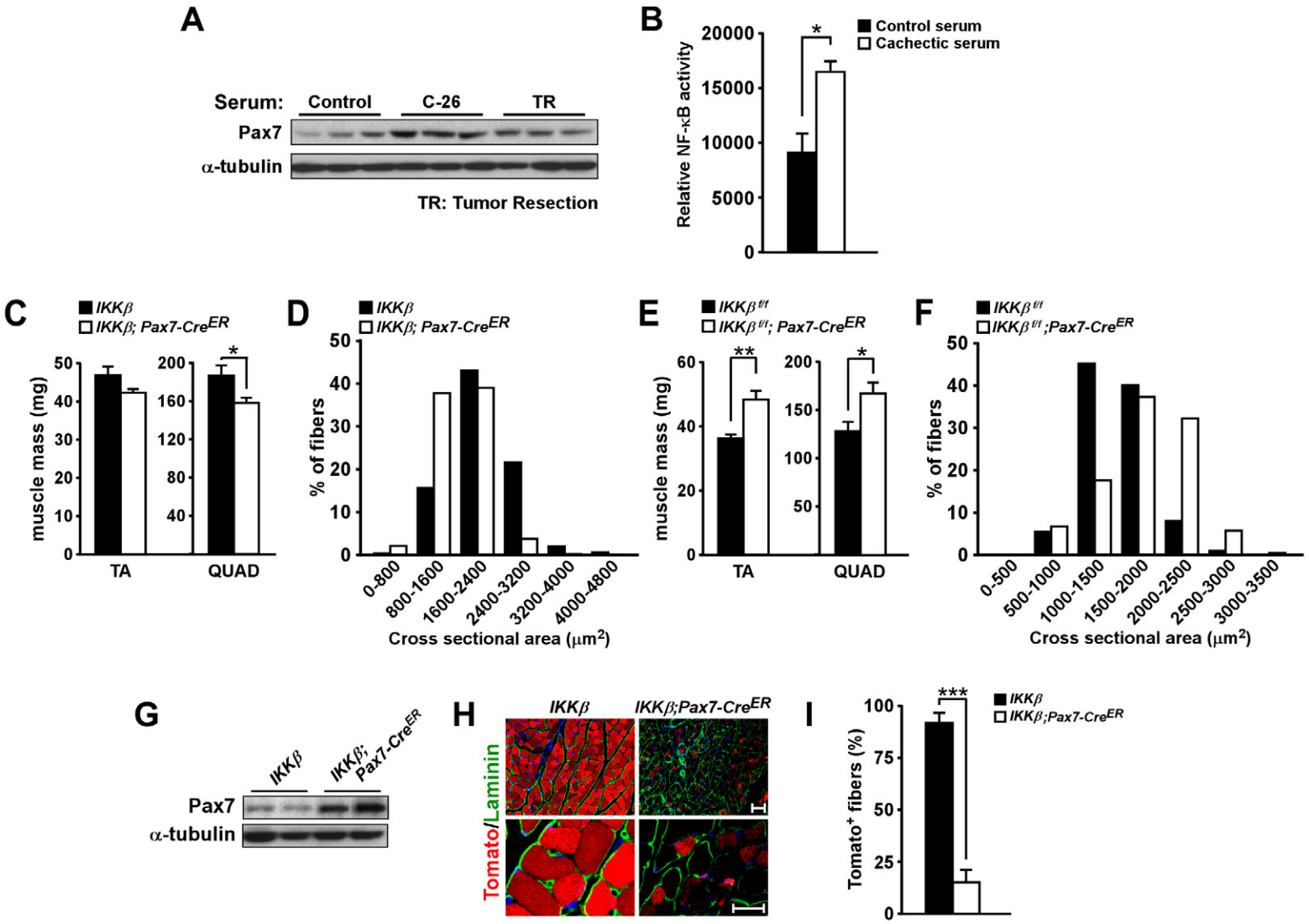

Figure S13. NF-κB signaling is activated by cachectic serum and regulates progenitor muscle cells to promote muscle wasting in cachexia. (**A**) Mouse serum collected from control, C-26 tumor and tumor resected (TR) mice were used to treat C2C12 cells, and Pax7 expression levels were analyzed by western at 24hr of treatment. (**B**) C2C12 myoblasts containing a stably expressing NF-κB luciferase reporter gene were incubated for 6hr with growth medium containing 5% control or cachectic serum isolated from C-26 tumor bearing mice. Luciferase values were normalized to total protein. Results were repeated in two independent experiments. (**C**) Graph shows TA and QUAD muscle masses from tumor bearing *IKKβ* (as control) or *Pax7-Cre^{ER}; Rosa26-IKKβ* mice injected with tamoxifen. (**D**) Cross sectional area measurements of fibers derived from GAST muscles from the same mice described in **C**. (**E**) Graph shows TA and QUAD muscle masses from tumor bearing *IKKβ$^{f/f}$* (as control) or *Pax7-Cre^{ER}; IKKβ$^{f/f}$* mice injected with tamoxifen. (**F**) Cross sectional area measurements of fibers derived from GAST muscles from the same mice described in **E**. (**G**) *Pax7-Cre^{ER}; Rosa26-IKKβ* mice at neonatal stage (P2) were injected with tamoxifen to induce NF-κB activation in muscle progenitor cells. Muscles were analyzed by western blot for Pax7 expression (*Rosa26-IKKβ* mice as control) at P16. (**H**) Cross sections of muscles from mice in **G** were visualized by Tomato reporter with α-Laminin immunostaining. Scale bars, 50μm (top) and 25μm (bottom). (**I**) Quantitation of Tomato^{+} fibers in **H**. Asterisks indicate $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).

Suppl. Table 1
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Patient Characteristics

Histology	Age	Sex	%wt loss	Pre-operative Chemotherapy	Figures
NC	47	M	0		1A, 2D, S2A
NC	27	M	0		2D
NC	59	F	0		2D
NC	27	N.D.	0		7B
NC	68	F	0		7B
NC	47	M	0		7B
PC	68	M	0	None	1A
PC	63	M	10	None	1A, 2D, 7B
PC	63	M	22	None	1A, 2D, 7B, S2A
PC	51	M	5	None	2D
PC	74	F	6	None	2D
PC	58	F	9	None	2D
PC	81	F	12	None	2D
PC	40	F	18	None	2D
PC	66	M	>20	None	2D
PC	50	M	N.D.	None	7B
PC	59	F	11	None	7B
PC	76	F	9	None	7B
PC	78	M	17	None	7B
PC	N.D.	M	11	None	7B(*)
PC	73	F	28	None	7B(*)
PC	82	M	7	None	7B(*)
PC	61	M	15	None	7B(*)
PC	66	F	14	None	7B(*)
PC	69	M	20	None	7B(*)

NC: Non Cancer Controls
PC: Pancreatic Cancer
M: Male F: Female
N.D.: Not Determined
(*): Patients included in the analysis, but data not shown.

Suppl. Table 2
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Primers used for RT-PCR and real-time PCR		
Gene	**Primer pairs**	**Size (bp)**
Pax7	5' - CCCTCAGTGAGTTCGATTAGCC - 3'	181bp
	5' - GGTCGGGTTCTGATTCCACA - 3'	
MyoD	5' - GAGCAAAGTGAATGAGGCCTT - 3'	328bp
	5' - CACTGTAGTAGGCGGTGTCGT - 3'	
Myogenin	5' - ATGGAGCTGTATGAGACATCCCC - 3'	237bp
	5' - CGACACAGACTTCCTCTTACAC - 3'	
PDGFRa	5' - CCTGTAACTGACACGCTCCG - 3'	247bp
	5' - CCATTTCCAAACCGCACA - 3'	
PW1	5' - ATGCCTCCTTTCTCACCG - 3'	181bp
	5' - ATGAGGACATTGGCTTCG - 3'	
NG2	5' - CTGTGCGTCGTTTGAGTTT - 3'	214bp
	5' - AGCGTAAGGGCTTTGGTC - 3'	
MuRF1	5' - ATTGTAGAAGCCTCCAAGGG - 3'	208bp
	5' - GGTGTTCTTCTTTACCCTCTGTG - 3'	
Atrogin-1	5' - AGATTCGCAAGCGTTTGATC - 3'	204bp
	5' - GGGAAAGTGAGACGGAGCAG - 3'	
Atg5	5' - ATCAGACCACGACGGAGCGG - 3'	116bp
	5' - GGCGACTGCGGAAGGACAGA - 3'	
Atg12	5' - ACAAAGAAATGGGCTGTGGAGC - 3'	195bp
	5' - GCAGTAATGCAGGACCAGTTTACC - 3'	
Beclin1	5' - TGAAATCAATGCTGCCTGGG - 3'	162bp
	5' - CCAGAACAGTATAACGGCAACTCC - 3'	
Bnip3	5' - CAGAGCGGGGAGGAGAAC - 3'	80bp
	5' - GAGGCTGGAACGCTGCTC - 3'	
Col1a1	5' - CCTGGACGCCATCAAGGTCTACTGC - 3'	164bp
	5' - ACTCGAACGGGAATCCATCGGTCAT - 3'	
Col5a1	5' - GCTCTTCGCTATTACCGATGCTTTC - 3'	243bp
	5' - AAACGGACTTGGCTCTGTTCCTCAC - 3'	
Col3a1	5' - AACCAGGTGCTAAAGGAGAAAGAGG - 3'	212bp
	5' - TGGATTACCATTATTGCCAGGAGGA - 3'	
Col5a2	5' - AAGCCTCGCAGAACCTTACTTACAT - 3'	264bp
	5' - TGCCAATATCCACAGGACCAACATC - 3'	
FN1	5' - GCAGTGGCTGAAGTCGCAAGGAAAC - 3'	258bp
	5' - ACCGCATGGTCTGTGCAGAAGGAAT - 3'	
GAPDH	5' - CACGGCAAATTCAACGGCACAGTCAAGG - 3'	252bp
	5' - GTTCACACCCATCACAAACATGG - 3'	

Suppl. Table 3
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Primary antibodies used for Western blotting					
Antibodies	**Clone**	**Host animal**	**Dilution**	**Source**	**Catalog**
Pax7		mouse	1:500	DSHB	
Desmin		rabbit	1:500	Sigma	D8281
MyoD		rabbit	1:500	Santa Cruz	sc-304
MyoD	5.8A	mouse	1:1000	DakoCyto	M3512
Myogenin		rabbit	1:500	Santa Cruz	sc-576
phospho-p38	28B10	mouse	1:1000	Cell Signaling	#9216
p38		rabbit	1:200	Santa Cruz	sc-535
α-tubulin	B-5-1-2	mouse	1:2000	Sigma	T5168

Secondary antibodies used for Western blotting			
Antibodies	**Dilution**	**Source**	**Catalog**
Anti-mouse IgG	1:250	Promega	W402B
Anti-rabbit IgG	1:250	Promega	W401B

Suppl. Table 4
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Primary antibodies used for Immunofluorescence					
Antibodies	**Clone**	**Host animal**	**Dilution**	**Source**	**Catalog**
Pax7		mouse	1:150	DSHB	
Desmin		rabbit	1:100	Sigma	D8281
Desmin	D33	mouse	1:100	DakoCyto	M0760
phopspho-Histone H3		rabbit	1:100	Cell Signaling	9701
Sca1	E13-161.7	rat	1:100	BD Pharm	553333
NG2		rabbit	1:100	USBiological	C5067-70D
PDGFRa	PDGFRa	rabbit	1:100	Santa Cruz	sc-338
Laminin	Laminin	rabbit	1:500	Sigma	L9393
BrdU	BrdU	rat	1:150	AbD Serotec	MCA2060
eMyHC	F1.652	mouse	1:500	DSHB	
Col IV		rabbit	1:500	Millipore	AB756P
MyHC (fast)	MY-32	mouse	1:500	Sigma	M4276

Secondary antibodies used for Immunofluorescence					
Host	**Against**	**Fluorophore**	**Dilution**	**Source**	**Catalog**
Goat	mouse IgG	Alexa Fluor 488	1:250	Invitrogen	A11029
Goat	mouse IgG	Alexa Fluor 568	1:250	Invitrogen	A11031
Goat	rabbit IgG	Alexa Fluor 488	1:250	Invitrogen	A11034
Goat	rabbit IgG	Alexa Fluor 568	1:250	Invitrogen	A11011
Goat	rabbit IgG	Alexa Fluor 350	1:250	Invitrogen	A11046
Goat	rat IgG	Alexa Fluor 488	1:250	Invitrogen	A11006
Goat	rat IgG	Alexa Fluor 568	1:250	Invitrogen	A11077
Goat	human IgG	Alexa Fluor 568	1:250	Invitrogen	A21090

Antibodies used for flow cytometry				
Antibodies	**Clone**	**mg/10^6 cells**	**Source**	**Catalog**
CD34-Alexa Fluor 647	RAM34	2	EBioscience	51-0341-82
Ly-6A/E(Sca1)-R-PE	E13-161.7	0.4	BD Pharmingen	553336
Ly-6A/E(Sca1)-FITC	E13-161.7	0.4	BD Pharmingen	553335
Integrin a7-FITC	3C12	10	MBL international	K0046-4
CD31-FITC	MEC 13.3	2	BD Pharmingen	553372
CD45-FITC	30-F11	0.4	BD Pharmingen	553080
CD11b-FITC	M1/70	0.4	BD Pharmingen	553310